Nutritional Challenges for Embryo Survival in Cattle

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Take-Home Message

Nutrition following the start of the breeding season, especially immediately after conducting artificial insemination (AI), is as critical as nutrition prior to breeding as it pertains to maximizing reproductive efficiency. Avoid exposing females to an abrupt change in nutrition during early gestation. Although the exact mechanisms that are causing reproductive failure are not known, it is clear that a sudden reduction in nutritional inputs immediately after insemination and during early gestation can result in impaired embryonic development and a reduction in pregnancy success.

Introduction

Numerous factors influence the probability of pregnancy success in cattle. Any management practice that detrimentally affects the ability of a female to conceive early in the breeding season ultimately detracts from reproductive and production efficiency. Often, nutritional management is the factor that greatest influences reproductive competence. Recently, the role of nutritional inputs following AI on the probability of conception in cattle has been investigated. The majority of these investigations have been conducted in the beef heifer, as common managerial practices may predispose these females to greater incidences of embryonic losses due to nutritional changes early in gestation. For instance, many spring-born heifers are developed from weaning to breeding in a dry-lot scenario and fed a diet consisting of a combination of forage and concentrate needed to gain approximately 1.5 lb. per day, targeting a final weight of 65% of estimated mature body weight at the time of breeding. Often estrous is synchronized and AI is conducted while in the dry-lot to better facilitate protocol implementation. Immediately following AI, heifers are often moved to pastures to expose them to clean-up bulls, take advantage of lush spring forage, and reduce the incidence of embryonic loss associated with handling and moving animals at later stages of early gestation. Such an immediate change in nutrition, due to shift in diet delivery method and/or quality and quantity of nutrients, may negatively impact metabolism, body weight gains, and reproductive efficiency in these heifers.

A potential mechanism by which a change in nutrition following insemination is effecting embryonic survival is by altering oviductal and uterine function. During early embryonic development, the developing conceptus is completely dependent upon secretions from the uterine endometrium, termed histotroph, to supply the nutrients necessary for conceptus growth and survival (Bazer, 1975; Bazer et al., 2009). Uterine histotroph is comprised of enzymes, cytokines, growth factors, ions, hormones, glucose, fructose, amino acids, transport proteins, and adhesion molecules (Bazer et al., 2012; Mullen et al., 2012). Many factors that are associated with metabolic status of an animal when measured in general circulation are also present in the histotroph during early gestation (Gao et al., 2009a,b). Thus, an immediate shift in

nutritional intake and an alteration in metabolic status may perturb uterine function and compromise embryonic development and maintenance of pregnancy.

The focus of this review is to summarize the current knowledge of how post-breeding nutrition is impacting embryo survival and pregnancy success and layout practical solutions to overcome reduced fertility due to nutritional changes during early gestation. Furthermore, this review will propose mechanisms by which nutritional inputs are affecting uterine function and embryonic development.

Post-insemination Changes in Nutrition on Embryonic Development and Pregnancy Success

Through decades of research, several efficacious estrous synchronization protocols exist for facilitating AI in beef heifers. Yet, across published reports and through practical experience, it is evident that sizeable herd-to-herd variation in heifer AI pregnancy rates still exists despite utilization of proven estrous synchronization and AI methodologies. This variation can only be partially attributed to semen source or pubertal status at initiation of synchronization. As such, it is evident that other aspects of heifer development and management are significantly contributing to the ultimate pregnancy success to AI. Often, heifers are developed in dry-lot conditions until the time of AI and then placed on spring pastures. This management approach exposes heifers to an abrupt nutritional change during early gestation. We believed that this apparent deficiency in current nutritional management of the beef heifer was impeding reproductive efficiency in the beef herd and conducted a series of studies to investigate how dietary changes immediately at insemination was impacting pregnancy success.

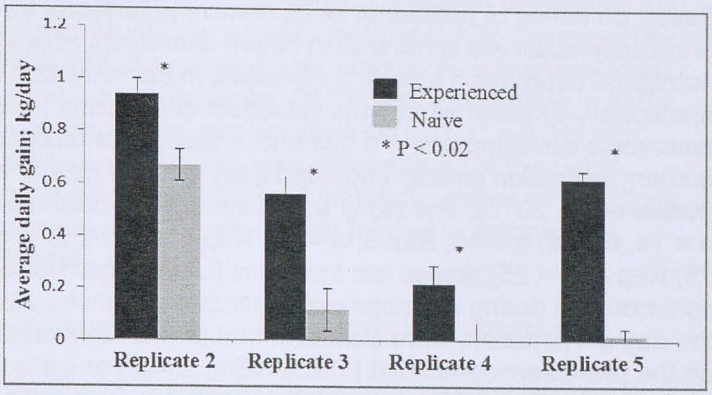
Development strategy, pasture introduction, weight changes, and AI pregnancy rates:

The original investigations in this line of research were conducted by Dr. George Perry at South Dakota State University (Perry et al., 2009, 2013). The objectives of these initial studies were to determine how previous grazing experience affected heifer growth and reproductive performance following estrous synchronization and AI. The initial study demonstrated that introducing beef heifers that were developed in a dry-lot scenario and unfamiliar with grazing (Naive) into pastures resulted in precipitous weight loss for the first 27 days compared to heifers that were acclimatized to grazing. Although specific nutrient quality of the pasture and daily feed intake of heifers was not evaluated in this study, it is estimated that weight losses experienced in the Naive heifers in this study would be equivalent to only consuming 40% of NEm requirements (Mackey et al., 1999).

The abrupt weight loss occurring following the transition from a dry-lot development scenario to pasture may be precipitating an abrupt shift in metabolic status and alterations in homeostasis to the heifers. It is accepted that a hierarchy exists amongst physiological systems in cattle in regards to nutrient utilization (Short et al., 1990). Therefore, it would not be surprising if an abrupt alteration in perceived nutrient status perturbed fertility, as nutritional inputs may be diverted from reproductive function to other physiological processes deemed more critical for maintenance of homeostasis. Therefore, Dr. Perry's group investigated if the transition from dry-lot to pasture at the time of AI affected AI pregnancy success. In 5 replications, beef heifers were either adapted to grazing prior to AI (Experienced; n = 207) or were placed on pastures immediately after AI with no prior grazing experience (Naive; n = 214). Similar to results of the previous experiment, heifers not accustom to grazing (Naive) had reduced ($P < 0.05$) average

daily gains from AI to pregnancy diagnosis (Figure 1; data for replicate 1 not available). This nutritional insult resulted in reduced ($P < 0.05$) AI pregnancy rates in the Naive (49.1%) compared to Experienced (59.4%) treatment. It is likely that the abrupt shift in metabolic status of heifers moved to pasture without prior grazing experience is directly responsible for this reduction in pregnancy success.

Figure 1. Average daily gain for Experienced and Naive heifers from AI to pregnancy determination



Controlled post-insemination nutrition and pregnancy rates:

Dr. Lake's laboratory at the University of Wyoming recently completed a controlled study to further investigate if an abrupt shift in nutritional intake following insemination resulted in reduced fertility in beef heifers (Arias et al., 2012; 2013). In this study, conducted over two years (2011 & 2012) at two locations per year (total of 4 replications), heifers were developed and maintained in a dry-lot scenario. Prior to AI, heifers were fed to achieve body weight gains to reach approximately 65% of mature body weight at the start of the breeding season. Following estrous synchronization and on the day of AI heifers were allotted to 1 of 3 post-insemination treatments to which heifers remained for 21 days. Post-insemination dietary treatments were: 1) 120% NEm (Gain), 2) 100% NEm (Maintain), and 3) 80% NEm (Lose). During the treatment period, dietary components were similar but NEm targets were achieved through limiting feed delivery. Although AI pregnancy rates did not differ between treatments (Table 1), contrast analyses revealed that AI pregnancy rates were reduced ($P = 0.05$) and breeding season pregnancy rates tended ($P = 0.106$) to be reduced in the treatments where NEm of diets were not adequate for continued growth (Lose and Maintain) compared to the Gain treatment (Table 1). Collectively, these data combined with data from Dr. Perry's laboratory strongly suggest that nutrient intake and body weight changes following insemination influence the probability of pregnancy to AI.

Table 1. AI and breeding season pregnancy rates in beef heifers fed to 120% (Gain), 100% (Maintain), and 80% (Lose) NEm following insemination.

	Treatment (Trt)				*P*-value	
	Gain (n = 118)	Maintain (n = 114)	Lose (n = 116)	Trt	Contrast: Gain vs Maintain + Lose	Contrast: Maintain vs Lose
AI pregnancy[1], %	72.9%	62.3%	64.7%	0.13	0.05	0.73
Breeding season pregnancy[2], %	94.1%	87.7	88.8	0.24	0.106	0.69

[1] Treatment x Replication, $P = 0.39$, thus replications combined for analyses.

[2] Treatment x Replication, $P = 0.65$, thus replications combined for analyses.

Post-insemination nutrition and embryonic development:

Based on timing of nutritional insult relative to fertilization, it is likely that pregnancy failure previously observed is not due to inherit alterations in oocyte quality. Rather, we propose that nutritional deprivation following AI results in the reduction in AI pregnancy success through nutritionally-induced alterations in oviduct and uterine function that are impacting normal embryonic development. To this end, a study was designed to determine if post-insemination nutrient restriction directly impacted early embryo quality and the number of live/dead cells (Kruse et al., 2013). The study was conducted in beef heifers in eight replications (Rep; Rep 1; n = 14, Rep 2; n = 15, Rep 3; n = 15, Rep 4; n = 14, Rep 5; n = 15, Rep 6; n = 15, Rep 7; n = 25, Rep 8; n = 25) across two locations (UMN; reps 1-6, SDSU; rep 7-8). All heifers were on a common diet during development. Estrus was synchronized and timed-AI was conducted. On the day of AI, heifers were placed in one of two nutritional treatments. Heifers either continued on the pre-insemination diet (120% NEm; GAIN) or were fed a sub-maintenance diet (LOSE; UMN, 80% NEm; SDSU, 50% NEm). At UMN, composition of the diet did not change between treatments but feed intake was limited to achieve targeted NEm. At SDSU, feed intake was not limited rather diet composition was altered to ensure both treatments had similar feed intake but differing NEm intakes. Dietary treatments were fed until single embryos were collected using non-surgical embryo flush techniques 6 days after AI. Recovered embryos were classified by developmental stage and graded (per IETS standards). Then embryos were transferred to the laboratory where number of dead blastomeres and total number of blastomeres was evaluated using epifluorescent staining. The lack of a treatment by replication interaction allowed data from all replications to be pooled for statistical analyses. Recovery rate of single embryo flushes did not differ between treatments (Table 2). Only 3 unfertilized ovum (UFO) were recovered. Visual embryo assessment by a trained embryologist blind to treatments revealed that embryo stage was less ($P < 0.01$) and embryo quality decreased ($P = 0.02$) in the LOSE compared to GAIN treatment. The number of total blastomeres and dead blastomeres were assessed via epifluorescent staining and the percentage of live cells per embryo calculated. In accordance with embryo stage results, total number of blastomeres within embryos in the LOSE treatment was decreased ($P = 0.03$) compared to embryos in the GAIN treatment. Although the number of dead cells did not differ between treatments, proportion of live cells within embryos was decreased ($P = 0.01$) in the LOSE compared to the GAIN treatment. In addition, accessory sperm number did not differ between treatments, indicating sperm transport was not affected by post-AI diet. Results indicated that embryo development in heifers receiving insufficient energy intakes following insemination was retarded within six days of administration of GnRH to induce ovulation and AI.

Table 2. Effect of post-insemination nutrition on day 6 embryo development[1]

					Blastomeres		
TRT	n[a]	% Embryos Recovered	Embryo Stage[b]	Embryo Quality[c]	Dead (n)	Total (n)	% Live
GAIN	46	46/65	4.6 ± 0.1	2.0 ± 0.2	7.8 ± 0.9	70.6 ± 5.6	83.3 ± 3.0
LOSE	42	42/66	3.8 ± 0.2	2.8 ± 0.2	9.7 ± 1.0	48.9 ± 3.9	71.1 ± 4.1
P-value	.	> 0.10	< 0.01	0.02	0.42	0.03	0.01

[1] Treatment by replication interaction not significant so data pooled across replications.

[a] Number of embryos evaluated.

[b] Stage of development (1-9;1 = UFO; 9 = expanded hatched blastocyst; per IETS Standards).

[c] Quality of embryo (1-5;1 = excellent; 5 = degenerate; per IETS Standards).

In the replications at UMN, circulating concentrations of progesterone and IGF-1 between AI and embryo collection were not affected by treatment. Hence, differences in embryo characteristics were not due to differing progesterone concentrations between treatments and were a direct effect of nutritional treatments. Moreover, across treatments IGF-1 concentrations at time of embryo recovery were not correlated to embryo stage, embryo development, total blastomeres, dead blastomeres, or percent live blastomeres. Of interest, the change in IGF-1 concentration between AI and embryo collection was correlated ($P < 0.001$; $r^2 = 0.31$) with embryo stage in the LOSE treatment, but this relationship was not observed in the GAIN treatment. Although IGF-1 is considered a general predictor of metabolic status, in this study IGF-1 was not altered by nutritional treatment and, in general, not related to embryonic characteristics between or across treatments.

Minimizing embryonic losses following insemination caused by a chance in nutrition:

The aforementioned studies demonstrate that: 1) an abrupt decrease in nutrient intake immediately following insemination results in a reduction of AI pregnancy rates, and 2) immediate alterations in early embryonic development are observed in heifers that fail to receive adequate nutritional inputs following insemination and these alterations in embryonic development are likely due to insufficient oviduct and uterine support of the developing conceptus. The obvious practical solution to this problem seems simple; don't expose heifers to nutritional insults following the start of the breeding season. Although this is critically important, managerial practices must be altered to accomplish this goal. Because in many ranching systems the initiation of the breeding season coincides with pasture turnout, leaving heifers in the dry-lot for an additional period of time following insemination is impractical and costly. Perry et al. (2009) demonstrated that heifers developed in dry-lot can be placed on pasture immediately after insemination if provided an additional concentrated feed. These authors demonstrated supplementing Naive heifers with a feed high in protein and energy (dried distiller's grains; 2.27 kg/d) prevented this reduction in AI pregnancy success (Naive; 61%, Naive + Supplement; 76%). In this study heifers were supplemented for 30 days following insemination. It has yet to be determined the duration of supplementation required to prevent a reduction in pregnancy success and this is the focus of future experiments. Another option is to expose heifers to pastures prior to insemination. Allowing heifers to become accustom to grazing and experience any weight loss prior to breeding can prevent the 'nutritional crash' from occurring simultaneously with breeding. This managerial strategy is, of course, dependent upon location, environment, and time of the breeding season.

Mechanisms by which Nutrition Influences Embryonic Development

Uterine secretions supporting embryonic development and facilitating conceptus elongation:

Based upon the aforementioned studies, it is likely that reduced pregnancy success in heifers not receiving adequate nutrition following insemination is due to an inability of the oviduct or uterus to maintain an appropriate microenvironment to support embryonic development. Unlike primates and mice, in cattle the attachment of the conceptus to the uterine endometrium and eventual placentation does not occur quickly after fertilization, but rather, the conceptus spends a prolonged period within the uterine lumen without a definitive attachment to the uterine endometrium. During this period the embryo is dependent exclusively on uterine secretions to provide an adequate microenvironment for continued development (Bazer, 1975; Bazer et al., 2009). These secretions are comprised of enzymes, cytokines, growth factors, ions, hormones, glucose, fructose, amino acids, transport proteins, and adhesion molecules (Bazer et al., 2012; Mullen et al., 2012), which are obligatory for embryonic development. While alterations in maternal nutrition have been demonstrated to alter the composition of follicular fluid and

influence oocyte competence (Leroy et al., 2008a), nutrient regulation of uterine secretions and subsequent embryonic development has not been completely elucidated. The ability of maternal metabolic imbalances to alter the uterine secretions of other species is suggested via recent investigations of nutritionally-induced alterations in the genome of the embryo via epigenetic modification (Seki et al., 2012; Ulbrich et al., 2013). Critical biochemical components of the uterine secretome include glucose, fructose, triglycerides, components of the IGF system, and amino acids but it is unclear if changes in global nutrition alter the amounts of these factors in the uterine lumen.

Glucose is a major energy substrate for the developing conceptus in ruminants (Bazer et al., 2011) and within the uterine lumen increase dramatically during pregnancy (Gao et al., 2009a,b). Furthermore, in cattle, advanced up-regulation of glucose transporters in the endometrium may contribute to increased embryonic development in cattle receiving progesterone supplementation (Forde et al, 2009, 2011). Fructose and tryglycerides also serve as energy substrates and facilitate embryonic growth and conceptus elongation (Ferguson and Leese, 2006; Kim et al., 2012). Differential expression in genes involved in triglyceride synthesis and secretion has been observed in the endometrium of heifers in which normal and developmentally delayed embryos were recovered (Beltman et al., 2010). Insulin-like growth factor-1 and -2, their complementary binding proteins, and receptors are present in the uterine endometrium, histotroph, and embryonic tissues. This system is critically involved in early embryonic development, participates in conceptus elongation, and regulates secretions from the uterine endometrium (Wathes et al., 1998; Webb et al., 1999). Amino acids serve numerous roles in biological systems and are components of enzymes, cytokines, hormones, and contribute to several cellular and metabolic functions (Groebner et al., 2011a). Limiting amino acid availability to the developing embryo can impede development (Steeves and Gardner, 1999). Of interest, Groebner et al. (2011b) observed differences in amino acid transport in somatic cell nuclear transfer (SCNT) derived embryos compared to *in vitro* produced embryos that may contribute to placental abnormalities previously observed with SCNT.

Whilst providing a uterine environment suitable for embryo survival, factors within the histotroph are also responsible for induction of mechanisms responsible for conceptus elongation. Without ample conceptus elongation during early gestation, the conceptus will fail to produce adequate interferon tau (IFNT) to signal maternal recognition of pregnancy (MRP; Anthony et al., 1988; Farin et al., 1990). Recently, the mechanistic target of rapamycin (mTOR) cell signaling pathway has been identified as a critical pathway for facilitating conceptus elongation in the ewe and pig (Gao et al., 2009f; Bazer et al., 2012). Given its functions in these species, it is likely involved in conceptus elongation in cattle as well. Although the mTOR pathway has not been investigated during embryo elongation in cattle, nutrient restriction at later stages of gestation in beef cattle has been demonstrated to alter the mTOR pathway in fetal tissue (Du et al., 2005). In the ewe, the mTOR cell signaling pathway is a nutrient sensing pathway that is activated by the presence of arginine and other selected amino acids, glucose, IGF-II, and phosphoprotein 1 (SPP1) in the histotroph (Kim et al, 2010, 2011; Bazer et al., 2011, 2012). A potential mechanism for reduced conceptus development in nutrient restricted heifers is alterations in the secretome that result in delayed or inactivation of the mTOR pathway.

Nutritional mediation of uterine function and embryonic development:

Few studies have directly addressed the impact of global under-nutrition on uterine functionality and directly related such findings to alterations in embryonic development. Wiebold (1988) reported differential amounts of glucose, total protein, calcium, magnesium, and potassium in the uterine flush media in lactating dairy cows where a normal versus abnormal embryo was collected 7 days after insemination. Beltman et al. (2010) compared the hormonal and metabolic

characteristics and endometrial gene expression profiles of beef heifers in which either a viable embryo or an embryo with reduced development was recovered on day 7 of gestation. Nine of 53 genes evaluated in the uterine endometrium differed between embryo classifications. Functions of the differentially expressed genes included immune response, signaling in the glycolysis pathway, aspects of prostaglandin synthesis, and triglyceride synthesis and metabolism. In the sow, reducing dietary intake to 0.6 daily maintenance energy requirement (NEm) following mating reduced embryonic survival, altered concentrations of nutritional metabolites and hormones in circulation and in the uterine lumen, and altered the expression of several genes involved in embryonic survival and nutrient transport and utilization (De et al., 2009; Xu et al., 2010). In the ewe, under-nutrition during early embryonic development delayed embryonic development on day 8 of gestation (Abecia et al., 1997) and increased embryonic mortality (Rhind et al., 1985, 1989; Abecia et al., 1999). Furthermore, conceptuses in undernourished ewes produced less IFNT and ewes had greater amounts of $PGF_{2\alpha}$ (Abecia et al., 1999).

Only two previous studies have evaluated the impact of nutrient restriction in heifers on fertilization and pregnancy rates (Hill et al., 1970; Spitzer et al., 1978). Hill et al. (1970) reported that when heifers were nutritionally suppressed (85% maintenance) for one estrous cycle prior to insemination, pregnancy rates were reduced compared to heifers fed to maintenance. These authors speculated that reduced fertilization rate in nutrient restricted heifers was the main reason for decreased pregnancy success. Conversely, Spitzer et al. (1978) concluded that breeding heifers at their second estrous cycle following severe nutrient restriction (33% maintenance) did not impact fertilization rates when assessed via surgical ova recovery conducted at 48 to 96 h post-AI. Rather, these authors concluded that reduced pregnancy rates in nutrient restricted heifers were due to embryonic death. Limited experimental units and confounding impacts of nutrient restriction on the oocyte limit conclusions that can be made from these studies, however both suggest that dynamic changes in nutrition can impact pregnancy success in cattle.

Conclusion

Although proper nutrition prior to the start of the breeding season is critical for optimizing reproductive efficiency in beef cattle, recent research highlighted above demonstrates that nutritional inputs following the start of the breeding program and in early gestation is also critically important. Most notably, avoid any abrupt changes in diet that can cause animal stress, alter metabolism, or result in sudden weight loss. Such occurrences can result in impaired embryonic development and ultimately a reduction in pregnancy rates.

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Notes

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